Claim 1 and dependent claims 5-10 were rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Examiner asserted that claim 1 and dependents 5-10 were unclear because the term "autologous" could only be defined relative to the individual into which it will be used, whereas the instant claims are drawn to a generic product. Applicants believe the use of "autologous" in the context of the claim, including as an intended use, will be readily understood as such by the artisan of ordinary skill in the art. To obviate the rejection, however, Applicants have deleted the term from claims 1 and 5-10. Based on the foregoing, withdrawal of the rejection under 35 U.S.C. §112, second paragraph is respectfully requested.

Claims 1-22 have been rejected under 35 U.S.C. 103(a) as allegedly obvious over the combination of Noishiki et al. (ASAIO Journal, 1992), Noishiki (US 5,171,261), and Zalewski (WO 93/15609, 1993). According to the Office, both Noishiki references describe the use of vascular grafts for seeding and deposition of vascular smooth muscle cells and endothelial cells. (Office Action, page 6.) The Noishiki references describe using heparin as an anti-clotting factor, but the heparin was quickly released from the graft and the grafts occluded due to an accumulation of thrombi.

In view of the quick loss of heparin from the grafts taught by Noishiki, Zalewski is relied on by the Examiner to allegedly provide a more long term solution to the treatment of vascular disorders. Zalewski is said to teach that vascular smooth muscle cells can be transformed with polynucleotides to express interferon, resulting in the inhibition of intra-vascular blockage, and that other transgenes can also be expressed, such as those encoding anticoagulants.

This rejection is respectfully traversed. It is not apparent how the teachings of Zalewski can be combined with Noishiki to remedy the clotting problems noted with the grafts of Noishiki. The two technologies approach different problems in very different ways. To solve the clotting problem of Noishiki one would have to ignore the majority of the teachings of Zalewski.

The Noishiki article is an operating room procedure to prevent or diminish graft occlusion. It describes using a segment of artery obtained from a dog in the operating room that

is then minced, incubated with heparin and seeded into the pores of a Dacron graft and immediately sewn back into the artery that provided the segment. The authors perform the whole procedure in about 15 minutes. The focus in on producing an anti-thrombogenic surface. The results are discussed with a view to demonstrating that thrombus formation is less in Dacron grafts seeded with tissue fragment plus heparin. There is a discussion of endothelialization of the graft surface as this could potentially provide a naturally non-thrombogenic surface. The cellular composition of the minced arterial suspension is not discussed. It is not obvious to someone skilled in the art that this would teach culturing of cells that comprise this tissue (either endothelial cells, vascular smooth muscle cells or adventitial fibroblasts) and their genetic manipulation to secrete therapeutic proteins. The emphasis is on thrombus formation in Dacron grafts that leads to their occlusion. The histology of graft sections removed from the dogs indicates that endothelial cells appear on the surface of the engrafted tissue, as well as vascular smooth muscle cells and fibroblasts. The discussion focuses on endothelial cells and the endothelialization of grafts from the anastomoses. This process occurs in non-seeded grafts but at a slower rate.

Neither of the cited Noishiki references teaches nor suggests the culturing of cells, their transduction and seeding into vascular grafts to provide therapeutic benefit. The emphasis of Noishiki's work is simply to provide a method that can be performed speedily in the operating room to cover Dacron prosthetic grafts with autologous minced artery to provide an antithrombogenic surface. The three cell types (endothelial cells, vascular smooth muscle cells and fibroblasts) composing the minced arterial tissue are not discussed in any way that would suggest their use as seeding cells, and it is not possible to culture these three cell populations together. Endothelial cells require growth components that are not ideal for vascular smooth muscle cells and this would prevent the transduction of all three cells in the tissue mix. Also, the large tissue fragments generated by vessel mincing would not enable efficient virus penetration and transduction of the mass of cells within the lumps. The central idea is to simply take the artery, mince it, and coat the resulting mixture onto the Dacron surface.

The Office Action states that "Zalewski et al. teach that vascular smooth muscle cells transformed with polynucleotides to express interferon result in the inhibition of intra-vascular blockage (summarized in the abstract)" Office Action page 7 lines 8-10. There are no

data in the Zalewski publication showing this, either *in vitro* or *in vivo*. The shopping list of potential therapeutic genes on page 3 does not teach that the underlying method will work for any of the genes. The dearth of data, both *in vitro* and *in vivo*, in the Zalewski publication makes the document read like a research plan. Zalewski proposes to denude the vessel wall and then assumes that vascular smooth muscle cells will be transduced by exposure to DNA-liposomes. No evidence supporting this assumption is provided in the publication. Also, there is no evidence that vascular smooth muscle cells treated in this manner express therapeutic genes over the long term. Also, we now know that the ability to achieve *in vivo* gene transfer to blood vessels by these methods is very limited and, to date, Applicants are not aware that such a method has provided gene therapy to anyone.

The Office Action suggests that one will transfect the smooth muscle cells according to Zalewski, for seeding in the vascular graft of Noishiki. Yet as discussed above, Zalewski is concerned entirely with *in vivo* infusion of vector. Zalewski describes denudation of the vessel wall before introduction of the reagents using mechanical means in combination with proteolytic enzymes (page 9 lines 8-13). The inflated balloon has apertures to deliver the reagents to the vessel wall. The publication does not discuss introduction of genetically modified cells using the device. The kits described in the invention are the catheter and mixtures of genes in pharmaceutically acceptable carriers with proteolytic enzymes, lipases and mild detergents. (Page 10 lines 21 -31). Genes are cDNA's in expression plasmids delivered as naked DNA or in liposomes (retroviruses are said to be too dangerous and not to be used (page 15 lines 27-29). There is nothing in the Zalewski publication which teaches the introduction of genetically modified vascular smooth muscle cells into a graft such as employed by Noishiki. (In all likelihood, the multiple apertures in a balloon catheter would become rapidly blocked by transduced cells if an attempt were made to deliver them to the injured vessel wall through the inflated catheter, as apertures of 10μm to 50μm (page 9 lines 27 -31) numbering 10 to 15 (page 10 lines 3-4) are taught). Also, the pressures described to inflate the catheter and deliver the reagents to the vessel wall (up to 5 atmospheres Page 19 lines 20-21) would be detrimental to the survival of primary vascular smooth muscle cells. And, *in vivo* transduction using plasmids or liposomes is not very efficient and given the short duration of exposure of the vessel wall to the transducing agent the number of transduced cells would be quite minimal.

The Zalewski PCT publication seems similar in some regards to the Nabel patent, over which rejections of the claims have been withdrawn. Nabel describes a double balloon catheter, and thus the devices of both Zalewski and Nabel focus on the instillation of reagents into the artery. (The Nabel device permits isolation and removal of blood products, permitting somewhat more efficient and attainable gene transfer.)

The Office Action states that given the guidance of both Noishiki and Zalewski there would have been a reasonable expectation of success to generate the instantly claimed graft. Given the absence of any data in the Zalewski patent and the crude mixed cell population in the Noishiki approach, the combined teachings would not even lead to the instant invention.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: September 25, 2002

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

1. (Thrice Amended) A device for implanting [autologous] vascular smooth muscle cells transduced with a gene of interest into a mammalian subject, comprising:

a tubular elongate member having a wall, which wall has an interior surface, an exterior surface, and pores therein; and

[autologous] smooth muscle cell transduced with the gene of interest immobilized within the pores and upon the interior surface of the wall to form a tubular smooth muscle cell complex whereby the smooth muscle cells remain stably immobilized on the graft surface and express a product of said gene.

5. (Twice Amended) The device of claim 1, wherein the [autologous] vascular smooth muscle cells are transduced with a gene encoding erythropoietin.

6. (Thrice Amended) The device of claim 1, wherein the [autologous] vascular smooth muscle cells are transduced with a gene encoding granulocyte colony stimulating factor or granulocyte macrophage colony stimulating factor.

7. (Thrice Amended) The device of claim 1, wherein the [autologous] vascular smooth muscle cells are transduced with a gene encoding Factor IX.

8. (Thrice Amended) The device of claim 1, wherein the transduced [autologous] vascular smooth muscle cells express an anticoagulant.

9. (Twice Amended) The device of claim 1, wherein the transduced [autologous] vascular smooth muscle cells are immobilized to the tubular elongate member with a polymer.

10. (Thrice Amended) The device of claim 1, wherein the device, prior to implantation in a subject, further comprises [autologous] vascular endothelial cells adherent to an interior surface of the tubular smooth muscle cell complex.